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DEPARTMENT OF THE ARMY  
Fort Detrick  
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# ISOCITRICODEHYDROGENASE ACTIVITY RETICULOCYTES AND NORMOCYTES

[Following is the translation of an article by F. Belfiore, G. Calceara, and S. Bonaventura in the Italian-language periodical Bollettino della Societa Italiana di Biologia Sperimentale (Bulletin of the Italian Experimental Biology Society), Naples, Vol 39, No 23, 1963, pages 1577-1580.]

From the Institute of Pathological Medicine of Catania University.  
Catania section -- 26 July 1963 session.

In earlier studies we investigated the behavior of some enzyme activities in the newly generated red blood corpuscles (reticulocyte) and in the mature red corpuscle (normocyte). We were thus able to observe that the morphologically reticulated young erythrocyte -- compared to the mature erythrocyte -- reveals an increase in malicodhydrogenase activity (1) as well as in glutamic-oxalacetic transaminase and glutamic-pyruvic activity and in arginase (2), glucose-6-phosphate dehydrogenase, phosphohexoseisomerase, aldolase, and lacticodhydrogenase (3), phosphoglycomutase, pyruvokinase (4), as well as in the ATP content (5) and the reduced glutathione content (6), while we did not observe any significant change in acid phosphatase (7) and in ATP-ase (4). We felt that this area of investigation was particularly useful, partly in connection with the knowledge of the enzyme picture of the red blood corpuscle during the first phases of its maturing evolution and partly because this line of investigation would enable us to arrive at a better evaluation of the enzyme changes in the erythrocytes under those morbid conditions accompanying a variation in the average age of the erythrocytes population; we therefore thought it a good idea to expand our research to other enzyme activities. In this report we took into consideration the behavior of isocitricodhydrogenase activity; we had one report (8) on this topic in the literature which indicates the essence of a modest amount of this enzyme activity in the reticulocytes.

Materials and methods. We took a group of 10 rabbits of both sexes and with an average weight of 1.5 kg; we treated this group with acetylphenyl-

hydrazine, administering a dose of 10 mg/kg/day subcutaneously for 5 days in order to induce an intense hemolytic crisis and, hence, a consecutive strong reticulocytosis which would enable us to study the newly generated erythrocytes. Before treatment and on the 7th, 12th and 20th days after the start of treatment, we took blood samples by means of cardiac puncture; in these blood samples we determined the number of red blood corpuscles per cu mm, the percentage of reticulocytes (using the method of supravital staining with brilliant-blue cresyl) and the isocitricodehydrogenase activity. This was determined according to the method of Wolfson and Williams-Ashman (9) on hemolysate prepared by adding 4 ml of distilled water to 1 ml of red blood corpuscles in physiological solution.

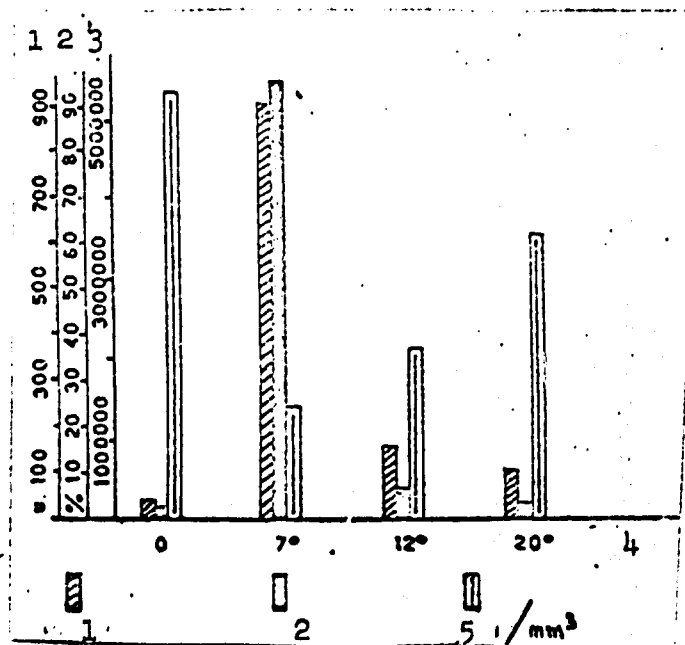
In order to determine whether the behavior of the enzyme activity was influenced by the toxic action of the hemolyzing substance used, we studied a second group of 10 rabbits in whom anemization had been induced by means of daily bleeding of an amount of 25 ml for 5 days.

The results are expressed in units and are related to 1 ml of hemolysate.

The data were worked out statistically.

Results: The data are shown in the attached figure; they tell us that as far as rabbits are concerned, the basic hematological conditions (erythrocytes 5,275,000 per cu mm, reticulocytes 2.7%), when the greater part of the erythrocytes is made up of mature elements, that is, normocytes, the erythrocytary isocitricodehydrogenase revealed an average activity of 38 U. On the 7th day of treatment, when we had maximum anemization (1,420,000 red blood corpuscles per cu mm) and when we had a marked increase in the reticulocyte rate, which went as high as 95%, we obtained a considerable increased value in the isocitricodehydrogenase activity corresponding to 893 U; this means that we obtained an increase of 1,980% in the normocyte activity, which is statistically significant ( $P < 0.001$ ).

On the 12th day, when the number of red blood corpuscles was 2,510,000 per cu mm and when the reticulocyte rate was 7%, the isocitricodehydrogenase activity had an average value of 146 U which gave us a 280% increase in the normocyte value ( $P < 0.001$ ). On the 20th day we had 3,523,000 erythrocytes, 2.5% reticulocytosis, and an average isocitricodehydrogenase activity of 100 U. Compared to the base activity, this would be an increase of 160% ( $P < 0.05$ ).



Isocitricodehydrogenase activity of erythrocytes in rabbit during experimental hemolytic anemia. Legend: (1) isocitricodehydrogenase; (2) reticulocytes; (3) erythrocytes; (4) days of treatment; (5) red blood corpuscles.

We always have a certain rate of nonreticulated mature elements even during the phase of maximum reticulocytosis (7th day); the activity of the reticulocytes is therefore quite obviously higher than the levels observed on the 7th day of experimentation. This activity, calculated with the help of a very simple procedure (1), gave us a figure of 904 U, with an increase of 2,290% with respect to the normocytes, taking into consideration the value of the mature erythrocytes which we know from the study conducted under basic conditions.

In the group of animals anemized by means of bleeding, we started from hematological conditions similar to those in the animals of the first group; in this second group we observed, on the 7th day, 2,593,000 red blood corpuscles, 41% reticulocytes, whereas we observed 2,900,000 red blood corpuscles per cu mm and 15% reticulocytes on the 12th day. This hematological situation is paralleled by an average isocitricodehydrogenase activity of 430 and 170 U, respectively; compared to the initial levels, those values represent an increase of 1,030% and 347%, respectively. Both of these changes are statistically significant ( $P < 0.001$ ); considering the different degree of reticulocytosis obtained in the two groups of animals, we can say that these changes are sufficiently similar to those observed in the first group of rabbits.

We also studied the correlation coefficient between the isocitricodehydrogenase activity and the reticulocyte percentage. This coefficient was

statistically significant ( $P < 0.001$ ). This proves that the course of isocitric dehydrogenase activity runs parallel to the course of the reticulocyte rate, and we must therefore conclude that the increase in this enzyme activity constitutes a characteristic of the young, morphologically reticulated erythrocyte. ( )

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